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Analysis of Microelectrode-Detected ESR (MEDESR) Signals of Nitroxide Radicals in Electrolyte Solutions

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Microelectrode-detected ESR (MEDESR) spectroscopy is a technique that is employed to observe magnetic resonance on the basis of an impedance change of electrodes. The characteristics of MEDESR for nitroxide radicals in electrolyte solutions were analyzed. MEDESR signals were obtained at zero bias-current, suggesting that modulation of the bias-current derived from ESR would not generate MEDESR signals. A good linear relationship between the MEDESR signal intensity and the applied power over a range of up to 150 mW was obtained. The frequency characteristics of the magnetic field modulation indicated that a simple thermal phenomenon would not cause an impedance change in MEDESR. When the radical was distributed on only the electrode surface, no signals were detected. The MEDESR spectra of a nitroxide solution, located outside the electrolytic cell, was detectable. These findings suggest that since MEDESR signals are irrelevant to the radicals in an electrical double layer, they are derived from some changes in the microwaves in the whole resonator. It is speculated, however, that the detecting mechanism of MEDESR is not a simple process of rectifying detection of a microwave change by resonant absorption like in conventional ESR, because the signal intensity of MEDESR is proportional to the irradiation power, and not to the square root as in the conventional ESR.

Electrically detected magnetic resonance (EDMR) spectroscopy is a technique used to observe the magnetic resonance on the basis of the electric change (such as an impedance change) of a sample. An EDMR determination method using semiconductors as samples has been developed from 1972.[1,2] It is well-known that EDMR has some advantages. First, this method is associated with high sensitivity (10^3 to 10^4 spins/10 mT). Second, the spins in the microregion (such as a *p*–*n* junction) are detectable with high selectivity.[1—4]

On the other hand, an electrochemical analytical technique using microelectrodes has also been employed for the quantitative analysis of a target substance existing in the microregion. This technique has extensively had good results in biological fields.[5—7] We think that the combination of EDMR spectroscopy and the microelectrode yields a new ESR technique. Microelectrode-detected ESR (MEDESR) applies EDMR to electrolytic solutions containing free radicals (model systems of a biological system). Recently, we detected the MEDESR signals of a stable nitroxide radical in a solution through a change in the potential between two electrodes inserted in the solution.[8] Here, the microelectrode (ϕ 50 μm) was biased by a constant-current source under microwave irradiation and a modulated magnetic field; and the AC component of the potential on the resonance was preamplified and detected by a lock-in amplifier.

A study is underway to arrive at a theoretical interpretation of the mechanisms of MEDESR. In this study, we have improved our MEDESR system to obtain data under various conditions. Using this system, the characteristics of MEDESR for the detecting mode, microwave power, frequencies of magnetic field modulation, and radical distribution were investigated. As a result, we have found that MEDESR signals were irrelevant to the radicals in the electrical double layer, and were derived from some changes in the microwave in the whole resonator.

Experimental

MEDESR System. Figure 1 shows a block diagram of our MEDESR system, which consists of an electrolytic cell, a bias-current/potential source and an AC preamplifier which were constructed in our laboratory, an X-band ESR spectrometer (modified TE200, JEOL) and an external lock-in amplifier (model 5210, PARC). The frequencies of the magnetic field modulation are variable from 80 to 640 Hz.

The bias-current source can be controlled in 0.1 nA steps, and the bias-potential source in 1 mV steps. The estimated noise magnitude of the standard voltage source was 20 $\mathrm{nV}/\sqrt{\mathrm{Hz}}$. The higher frequency components (> 10 Hz) were reduced through a passive low-pass filter. The standard voltage source, a low-pass filter, a variable resistor, and a buffer constitute a presettable voltage source. The accurate, low-noise property of the bias-current/potential source enables reproductive measurements.

The AC preamplifier must satisfy three conflicting requirements: low noise amplification, high input impedance, and flat response over a wide frequency band. To reduce noise, close shielding and a large gain in preamplification are effective. However, close

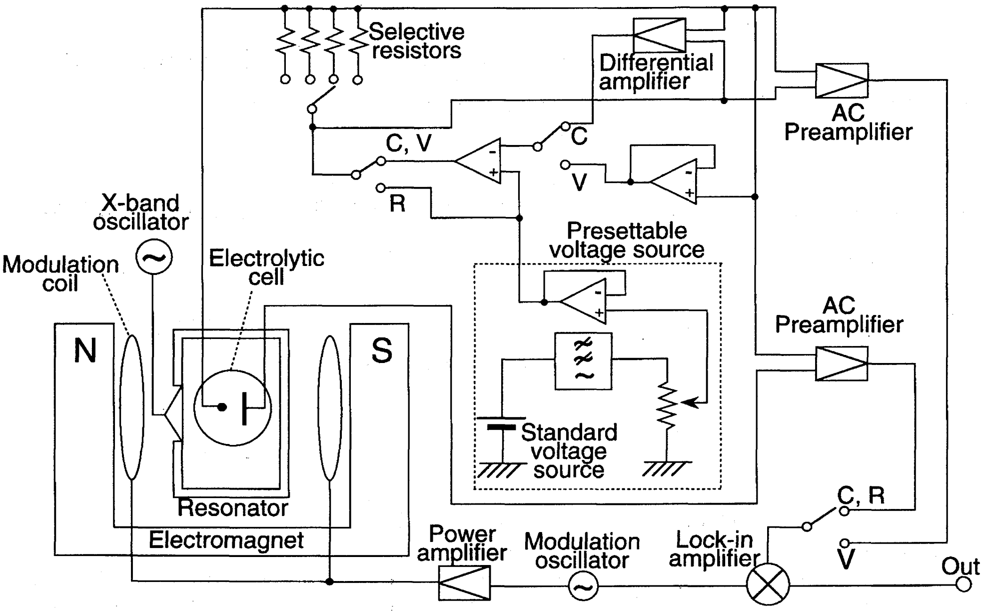

Fig. 1. Block diagram of our MEDESR system.

shielding increases the capacitance of the input impedance; also, the combination of a large gain and high impedance readily causes undesirable oscillation through a very small stray capacitance. Amplification over a wide frequency band increases the noise level, and also easily generates undesirable oscillation or an uneven frequency response, especially when feedback is applied to control the bias-current/potential. To satisfy these requirements and to overcome the problem, the circuit design, devices used, and their layouts were carefully investigated. The input impedance of the AC preamplifier, including cables, was 9×10^{11} Ω of resistance and 1 nF of capacitance in parallel. The gain was 40 dB and the bandwidth at the -3 dB points was 5 kHz.

The impedance changes between electrodes are detectable by three modes: constant-current potentiometric (C), constant-voltage amperometric (V), and constant-resistance potentiometric (R) modes. Although the C-mode matches the high impedance of the microelectrode, it has a disadvantage: the potential, which controls the redox reaction, drifts during measurements taken over a long time. The V-mode has an advantage, i.e., a stable potential; however, it is difficult to design a detecting system because of the very small changes in small currents at the microelectrode. In the R-mode, one of the selective resistors is directly connected to the presettable voltage source without feedback. Nonfeedback guarantees that the detecting system will have flat frequency characteristics. The disadvantage due to potential drifting is similar to what happens in the C-mode. Switching from one mode to another is easily and quickly accomplished.

The electrolytic cell was made of a glass capillary tube (1.1 mm in inner diameter, 15 cm in length), a working microelectrode, and its reference electrode. The working electrode was located at the center of the resonator. An Ag/AgCl electrode, located outside of the resonator, served as a reference electrode. It was connected to the electrolytic cell through a salt bridge (Fig. 2a).

Materials. Nitroxide radicals, 4-hydroxy-2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPOL) and 4-amino-2,2,6,6-tetramethylpiperidin-1-oxyl (amino-TEMPO) were purchased from Aldrich Chem. Co.

The microelectrode was made as follows. Gold wire (50 μm

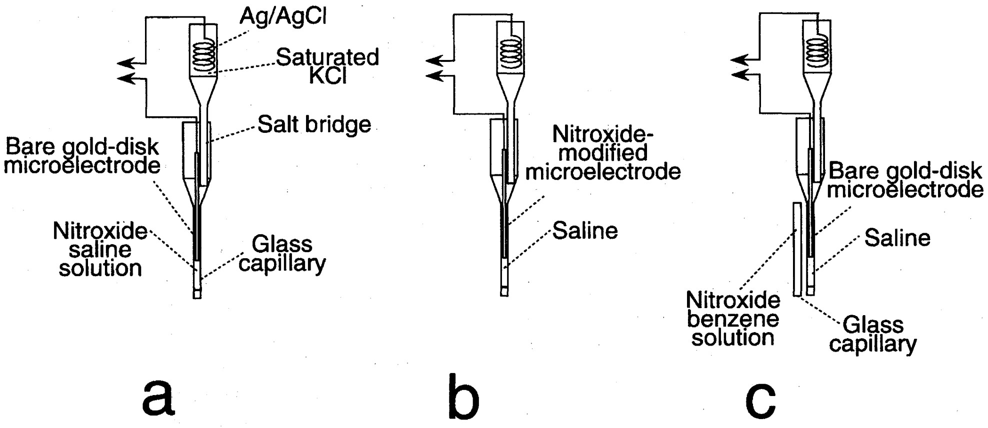

Fig. 2. Structure of the electrolytic cell. (a): MEDESR measurements of nitroxide saline solution that is placed in the electrolytic cell, using the bare gold-disk microelectrode. (b): MEDESR measurements of the nitroxide-modified microelectrode in saline that is placed in the electrolytic cell. (c): The bare gold-disk microelectrode is placed in a saline solution which was put in the electrolytic cell; and a glass capillary tube containing nitroxide benzene solution is placed next to the electrolytic cell.

in diameter), which was connected to a copper wire by electric welding, was insulated by melted lead glass, and then enclosed into a glass capillary tube (1 mm in outer diameter) by using epoxy resin. The tip of the electrode was polished to make a flat surface to finish a bare gold-disk microelectrode.

The bare gold-disk microelectrode was immersed in a 10 mmol dm^{-3} DL-α-lipoic acid (Wako Pure Chemical Industries Ltd.) methanol solution for 24 h. After being cleaned through sonication in pure water, the electrode was immersed in an aqueous mixture of 100 mmol dm^{-3} amino-TEMPO and 100 mmol dm^{-3} 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide (Wako Pure Chemical Industries Ltd.) for 6 d. During this process, the nitroxide was immobilized on the surface of the gold-electrode.[9] Following sonication in pure water, the electrode was used as a nitroxide-modified microelectrode.

Results and Discussion

Detecting Mode Characteristics. Using the bare gold-disk microelectrode, we conducted MEDESR measurements of a 1 mol dm^{-3} TEMPOL saline solution that was placed in an electrolytic cell (Fig. 2a). MEDESR signals were obtained in all three modes, viz., C-, V-, and R-mode (Fig. 3). The signal-to-noise ratios (SNRs) derived from the C- and R-modes were nearly the same. The SNR in the V-mode was lower than those in the other modes.

When the resistance was set at 500, 50, 5, or 0.5 MΩ in the R-mode, a sharp drop in the signal intensity (peak-to-peak height) at 0.5 MΩ was observed. This suggests that the signal impedance is a few MΩ. This value is bigger than the almost zero input impedance of the whole detecting system in the V-mode ($\ll 1$ Ω), being consistent with the low SNR in the V-mode compared to the other modes. It is also smaller than that of the direct-current resistance between the electrodes (from dozens of MΩ to several GΩ).

When the bias-potential between electrodes changed by about 0.15—0.4 V in cases where the nitroxide was not almost oxidized, changes in the signal intensity were rarely observed. Under these conditions, the bias-current ranged from approximately 0 to 10 nA. Because the MEDESR signal was obtained at zero bias-current, the hypothesis that modulation of the bias-current derived from ESR would produce MEDESR signals was not substantiated.

Microwave Power Characteristics. Figure 4 shows the signal intensities plotted against the power levels of irradiation. The electrode and sample were similar to those already described. A good linear relationship between the MEDESR signal intensity and the applied power was observed over the range up to 150 mW. At 200 mW, the signal intensity deviated from linearity because of the saturation of ESR irradiation. This linear power characteristic below saturation is similar to that of the EDMR signals of from a *p–n* junction of a semiconductor.[10]

Modulation Frequency Characteristics. Figure 5 shows the signal intensities plotted against the modulation frequency. The electrode and sample were similar to those already described. The signal intensity increased once with the modulation frequency up to 320 Hz, and was then followed by a decrease at 640 Hz. If a change in tempera-

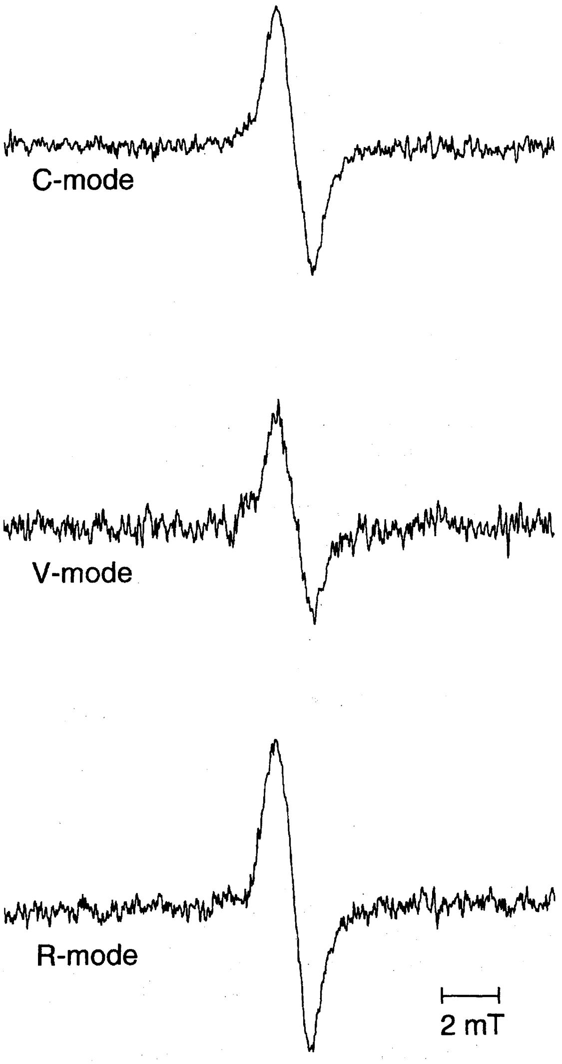

Fig. 3. MEDESR spectra of 1 mol dm^{-3} TEMPOL saline solution at C-, V-, or R-mode. The instrument settings are: microwave power, 200 mW; microwave frequency, 9.5393 GHz; scan rate, 0.3 mT s^{-1} accumulation number, 16; modulation frequency, 160 Hz; time constant, 30 ms; bias-current, 10 nA (C- and R-mode), bias-potential, 370 mV (V-mode).

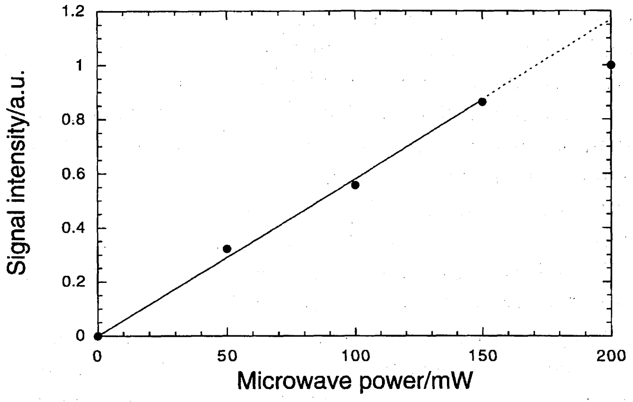

Fig. 4. Signal intensities of MEDESR spectra obtained from a 1 mol dm^{-3} TEMPOL saline solution plotted against the power level of irradiation. Instrument settings are similar to those described in Fig. 3.

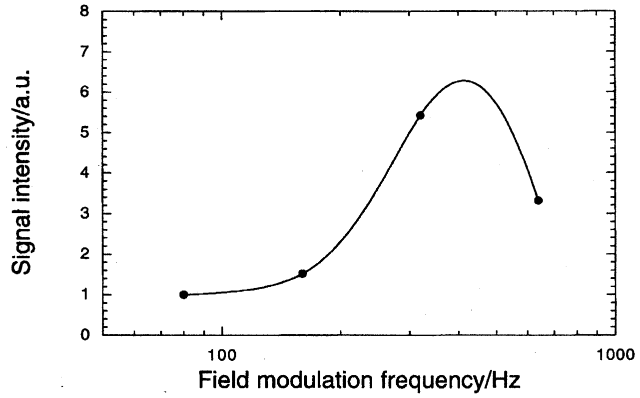

Fig. 5. Signal intensities of MEDESR spectra obtained from a 1 mol dm^{-3} TEMPOL saline solution plotted against the frequency of the magnetic field modulation. Instrument settings are similar to those described in Fig. 3.

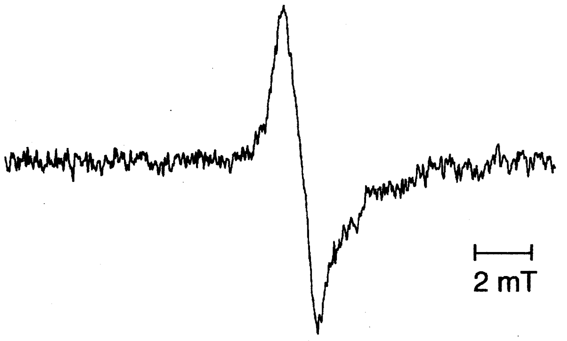

Fig. 6. MEDESR spectrum (C-mode) of 1 mol dm^{-3} TEMPOL benzene solution that is located outside the electrolytic cell. For the details of the electrolytic cell, see Fig. 2c. Instrument settings are similar to those described in Fig. 3.

ture caused by the ESR absorption causes a change in the impedance in the MEDESR measurements, the signal intensities would be reduced in correspondence with increasing modulation frequency, where heating/cooling cannot follow. Thus, MEDESR is apparently not just a simple thermal phenomenon.

Radical Distribution. MEDESR measurements of a nitroxide-modified microelectrode in a saline solution, which was put in an electrolytic cell, were conducted (Fig. 2b). Under this condition, the radicals only distributed on the electrode surface. However, no signals were detected.

A bare gold-disk microelectrode was inserted into a saline solution, which was put in the electrolytic cell; then a glass capillary tube (1.1 mm in inner diameter, 15 cm in length) containing 1 mol dm^{-3} TEMPOL benzene solution was placed next to the electrolytic cell (Fig. 2c). Here, TEMPOL was dissolved in benzene so as not to reduce the Q-factor. The electrode did not come into electrical contact with the radical solution. When the MEDESR measurement was conducted under these conditions, as shown in Fig. 6, we obtained an MEDESR signal that resembled that obtained when a bare gold-disk microelectrode was immersed in a radical solution (Figs. 3a, 3b, and 3c). These findings indicate that MEDESR signals are not generated by radicals located on the surface of an electrode. Especially, the fact of signal detection from the electrical isolated radical suggests that MEDESR signals are irrelevant to the electrical double layer, and are based on a fundamentally different principle.

Because an electrolyte is highly conductive, and the electrical double layer has a high resistance, the impedance between two electrodes almost reflects that of the electrical double layer. Therefore, in a previous study, we thought that the MEDESR signal would be selectively derived from the electrical double layer; thus, the sensitivity of MEDESR was estimated to be about 5×10^6 spins/10 mT based on the volume of the electrical double layer.[8] The results of the present study negate this assumption. They suggest that some changes in the microwave in the whole resonator produce the MEDESR signals. This probability, together with the above-mentioned finding, i.e., low signal impedance compared with the direct-current resistance, is not inconsistent with the speculation that a diode-like device is a signal source of MEDESR. However, we do not believe that the detecting mechanism of MEDESR is a simple rectifying detection of the microwave change by resonant absorption such as seen in conventional ESR, because the signal intensity of MEDESR is proportional to the irradiation power, and not to the square root as in the conventional ESR. Therefore, the MEDESR technique has potential to supply a new microwave-detecting device that is different from a conventional detector diode.

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9) To confirm immobilization of the nitroxide on the surface of the gold-electrode, the same treatment gave a thick and long gold wire (0.3 mm in diameter, 30 mm in length). ESR spectrum of the nitroxide from this gold wire, obtained by using a conventional ESR spectrometer, is a broad singlet signal caused by poor mobility of the nitroxide on the surface of the gold wire.

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